

The resulting CH_2Cl_2 solution was filtered to remove the NH_4Br and concentrated, and the residue was recrystallized from 2 l. of cyclohexane to give 19.4 g. of product, m.p. 133–135°. The product appeared to contain cyclic XII as reflected in the analysis.

Anal.—Calc. for $\text{C}_{15}\text{H}_{11}\text{ClF}_2\text{N}_2\text{O}_2$: C, 55.48; H, 3.42; Cl, 10.92; F, 11.70; N, 8.63. Found: C, 56.69; H, 3.99; Cl, 11.19; F, 11.06; N, 8.34.

1,3-Dihydro-5-(2,6-difluorophenyl)-7-chloro-2H-1,4-benzodiazepin-2-one (XII)—A solution of 21.0 g. (0.065 mole) of XI in 300 ml. of pyridine was heated under reflux under N_2 for 18 hr. The reaction mixture was concentrated and the residue was washed with Skellysolve B and recrystallized from ethyl acetate–Skellysolve B and then ethyl acetate to give 11.7 g. of product in Crop 1, m.p. 248–249°, and 2.3 g. in Crop 2, m.p. 244–246°. This material was found to contain solvated ethyl acetate; therefore, an additional recrystallization of a 10-g. sample from EtOH was performed to give 7.0 g. in Crop 1, 1.3 g. in Crop 2, and 1.0 g. in Crop 3, m.p. 251–253°.

Anal.—Calc. for $\text{C}_{15}\text{H}_9\text{ClF}_2\text{N}_2\text{O}$: C, 58.74; H, 2.96; Cl, 11.56; F, 12.39; N, 9.14. Found: C, 58.84; H, 2.89; Cl, 11.56; F, 12.01; N, 9.11.

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Characterization of Sulfonamides by TLC Using Metal Ions[†]

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Abstract □ The reactions of metal ions such as copper (II), cobalt (II), nickel (II), and cerium (IV) with 15 sulfonamides on TLC plates were examined to obtain a suitable reagent for the visualization of these drugs. A solution of copper acetate in methanol produces spots of varying colors, whereas an acidic solution of ceric sulfate gives yellow or purple spots with all the sulfonamides studied. Cobalt (II) and nickel (II) are specific for a limited number of sulfonamides.

Keyphrases □ Sulfonamides—characterization by TLC, using metal ions □ TLC—characterization of 15 sulfonamides using metal ions

Various chromatographic procedures have been proposed for the separation and identification of sulfonamides. The literature on such methods employing TLC was recently reviewed (1, 2). *p*-Dimethylaminobenzaldehyde, coupling with *N*-(1-naphthyl)ethylenediammonium dichloride after nitrous acid treatment, vanillin, and 1-phenyl-3-methyl-2-pyrazoline-5-one in pyridine with potassium cyanide after chlorine treatment have been generally employed as locating agents for sulfonamides. These are either nonspecific, because they give colors with other compounds containing the same func-

tional groups, or are unable to distinguish between various sulfonamides. The use of metal ions as characterizing agents for sulfonamides has not been studied in detail. Güven and Pekin (3) used an ammoniacal solution of copper and, more recently, Clarke and Humphreys (4) employed aqueous copper sulfate as a visualizing agent for sulfonamides on alkaline TLC plates.

The present study describes the reactions of 15 sulfonamides on chromatoplates with several metal ions such as copper (II), nickel (II), cobalt (II), and cerium (IV) in an attempt to evolve a suitable agent for characterizing individual sulfonamides by different color spots.

EXPERIMENTAL

Materials—Pure drug samples were obtained from various pharmaceutical companies. Silica gel F 254 was used¹. Metal salts, solvents, and other reagents were analytical grade.

TLC Plates—The 20 × 20-cm. glass plates were coated with a 250- μm . layer of silica gel slurry in water and dried for 1 hr. at 110°. The dried plates were cooled to room temperature before use.

¹ E. Merck, Germany.

Table I—Colors Obtained by Treating Sulfonamides with Metal Ions

Sulfonamide	R_f Value	Colors with Locating Reagent ^a					
		I	II	III	IV	V	VI
Sulfanilamide	0.61	Faint green	—	—	—	Yellowish purple	—
Sulfadiazine	0.56	Brown	Brown	Brown	—	Yellow (tailed)	—
Sulfamethazine	0.65	Yellowish green	—	Orange	—	Yellowish purple	—
Sulfamerazine	0.63	Brown	Light brown	Brown	—	Yellowish purple	—
Sulfacetamide	0.54	Faint yellow	—	—	—	Yellow	—
Sulfamethoxy-pyridazine	0.62	Brown	Reddish brown	Light brown	—	Yellowish purple	—
Sulfisomidine	0.50	Yellow	Light green	Light yellowish green	—	Yellow	—
Sulfapyridine	0.63	Reddish brown	Brown	Light brown	—	Yellowish purple	—
Sulfadimethoxine	0.67	Yellowish green	Yellowish green	Yellow	—	Yellowish purple (tailed)	—
Sulfathiazole	0.52	Pink	Reddish brown	Light purple	Reddish brown	Purple	Brown
Sulfamethizole	0.54	Yellowish green	Light yellow	Yellowish green	—	Purple	Light yellow
Sulfaethidole	0.58	Yellow	Light yellow	Faint yellow	—	Purple	Light yellow
Sulfisoxazole	0.63	Faint yellow	—	Light brown	—	Purple	—
Sulfamethoxazole	0.59	Yellowish green	Yellowish green	Yellowish green	—	Purple	—
Phthalylsulfathiazole	0.11	Purple	Purple	Light purple	—	Purple	—

^a The roman numerals refer to the locating reagent described under *Experimental*.

Standards—A 10-mg. quantity of pure drug was dissolved in 1 ml. of solvent (10% concentrated ammonia solution in acetone).

Solvent System—A mixture of ethyl acetate (90 ml.) and methanol (10 ml.) was used to saturate the chromatographic tank (21 × 21 × 10 cm.) and to develop the plates. The ends of the tank were lined with filter paper for saturation. The solvent system was prepared fresh for each run.

Locating Reagents—The following locating reagents were freshly prepared for use: I, copper acetate in methanol (saturated solution); II, copper acetate in acetone (saturated solution); III, copper sulfate (5%) in water; IV, cobaltous nitrate (2%) in water; V, ceric sulfate (2%) in water using 5 ml. concentrated sulfuric acid; and VI, nickel chloride (2%) in water.

Method—One microliter of the sulfonamide solution was applied to the TLC plate and dried in a current of hot air. In most experiments, all 15 samples were applied on a single plate. The developing tanks were saturated with the solvent mixture for 1 hr. before initiating the development of the chromatoplate. After the development had taken place to a height of 15 cm., the plates were taken out and air dried before spraying with the locating agent.

When visualization was accomplished with aqueous copper sulfate, the plates were sprayed with 0.1 *N* sodium hydroxide and dried prior to treatment with the reagent.

RESULTS AND DISCUSSION

The colors obtained with various metal ions are given in Table I along with the R_f values. Nickel and cobalt salts gave colored spots with relatively few sulfonamides. Sulfathiazole, sulfamethizole, and sulfaethidole were detected by the nickel chloride solution. The cobalt nitrate solution specifically characterized sulfathiazole. All of the sulfonamides studied were oxidized by ceric sulfate, resulting in yellow, yellow-purple, or purple spots. The range of colors obtained was much broader with copper salts.

Both aqueous copper sulfate and saturated copper acetate in

methanol gave similar colors in most cases; however, the latter should be the reagent of choice because the spots obtained are brighter and, due to the volatile nature of the solvent, the plates can be dried quickly. Sulfanilamide and sulfacetamide, which could not be seen by aqueous copper sulfate, appeared as green and yellow spots, respectively, with copper acetate in methanol. Phthalyl-sulfathiazole gave two spots, a major spot which hardly moved from the point of application and another small pink spot which was probably N-4 unsubstituted drug (sulfathiazole).

The colors obtained from methanolic copper acetate do not fade and are stable for several days. The present screening with different metal ions offers suitable locating reagents for rapid identification of sulfonamides on TLC plates. The characterization is easily done from the colors obtained by reaction of the drug and the metal ion.

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